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Oxyphenisatin Derivatives and Intestinal Glucose and Tyrosine Absorption

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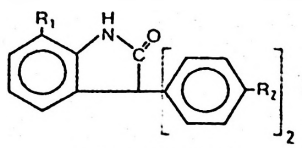
The effect of oxyphenisatin and three other isatin derivatives on glucose and tyrosine absorption is studied in rat small intestine, *in vitro*. Compounds with free phenolic groups elicit, even at low concentrations, strong inhibition on active transport, while those with the sulfate-esterified phenolic groups show no effect whatsoever. One minute preincubation with 10^{-4} M oxyphenisatin is enough to inhibit sugar absorption completely.

The inhibitory effect that polyphenolic compounds such as phloretin, phenolphthalein and oxyphenisatin exert on the intestinal absorption of sugars and amino-acids is well known both *in vivo* and *in vitro* (1, 5, 6). In recent years, new isatin derivatives have been introduced as laxatives in therapy. One of these new compounds, sodium sulisatin, has been found to be incapable of inhibiting glucose absorption *in vivo* (8). The present study has been designed to examine the effect of four structurally related compounds, with laxative activity, on the glucose and tyrosine absorption *in vitro*.

Materials and Methods

Experiments were carried out with sacs of everted small intestine of male Sprague-Dawley rats (220-270 g), as described by WILSON and WISEMAN (9), and the middle ileum was used in all cases. Glucose (2 mM) or tyrosine (1 mM) was placed in both mucosal (20 ml) and serosal (1.5 ml) sides. Compounds studied, oxyphenisatin, sodium sulphatin, bis-(p-hydroxyphenyl)-7-methyl-2-indolinone (BHMI) and sodium sulisatin (table I) were placed in the mucosal solution. Some experiments with oxyphenisatin in the serosal or with preincubation with this compound in the mucosal were also done. After a 60-min incubation period final serosal and mucosal concentrations of glucose or tyrosine were determined by the methods of HULTMANN (7) and CERIOTTI and SPANDRIO (3), respectively.

Table I. *Effect of four indolinone derivatives on the final serosal/mucosal concentration ratio of glucose and tyrosine, in everted sacs of rat ileum.*
The incubation period was of 60 min at 37° C in carbogen atmosphere. The initial volumes were 20 ml for the mucosal and 1.5 for the serosal. The starting concentration of glucose was 2 mM and of tyrosine of 1 mM, in both serosal and mucosal compartments. n: number of experiments. Significances: * $p < 0.05$; ** $p < 0.001$ (Student's t test).



BASIC STRUCTURE

	ISATIN DERIVATIVE	Conc. (M)	GLUCOSE		TYROSINE	
			n	serosal/mucosal ratio	n	serosal/mucosal ratio
	None	—	56	2.26	40	3.23
R_1 : H, R_2 : OH	Oxyphenisatin	10^{-7}	7	1.48*	—	—
		10^{-5}	8	0.74**	8	1.15**
		10^{-3}	4	0.68**	4	0.91**
R_1 : H, R_2 : OSO_3Na	Sodium sulphatin	10^{-5}	7	2.36	4	3.15
		10^{-3}	4	2.23	8	3.16
R_1 : CH_3, R_2 : OH	BHMI	10^{-5}	4	0.96**	4	1.00**
		10^{-3}	8	0.78**	4	1.03**
R_1 : CH_3, R_2 : OSO_3Na	Sodium sulisatin	10^{-5}	11	2.25	4	3.35
		10^{-3}	10	2.10	4	3.21

Results and Discussion

The serosal to mucosal final concentration ratio (S/M ratio) of glucose or tyrosine, for each compound studied, is indicated in the table. Oxyphenisatin and BHMI strongly inhibit glucose and tyrosine transport since the S/M ratios are in both cases near to or lower than 1, while in the controls this ratio is higher than 2 for glucose and higher than 3 for tyrosine. On the other hand sulphatin and sulisatin show no effect on glucose and tyrosine absorption at any of the concentrations tested. The strong inhibition exhibited by oxyphenisatin is much higher than that found by BIANCHETTI and GIACHETTI in the guinea-pig ileum (2) and by HART and MCCOLL in rat small intestine *in vivo* (6) even at lower concentrations than used by these authors. Glucose absorption appears to be completely suppresed either with oxyphenisatin or BHMI. The final glucose serosal concentration is lower than the initial one and this may be attributed to a serosal to tissue downhill concentration gradient (figure). With oxyphenisatin the effect is similar in both 10^{-5} and 10^{-3} M concentrations while in the case of BHMI it appears that the higher the concentration the higher the μmol which leave the serosal space. With tyrosine there is not significant disappearance of this aminoacid from the serosal compartment, probably due to the fact that this substance is not metabolized for energy requirements by the intestinal cells.

In preincubation studies with oxyphenisatin, for a duration of 1 or 5 min, a strong inhibition of glucose active transport was observed. The S/M ratios were reduced from 2.02 (controls) to 0.81 (1 min preincubation) and 0.82 (5 min preincubation) both values being statistically different from the controls ($p < 0.001$). The preincubation experiments confirmed the

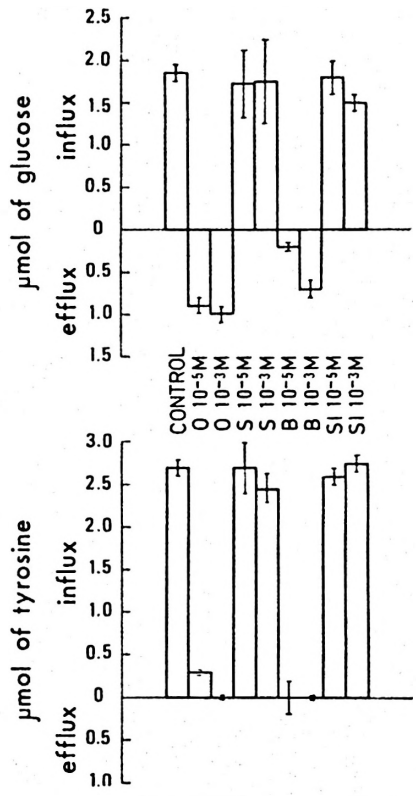


Fig. 1. *Effect of oxyphenisatin (O), sulphatin (S), BHMI (B) and sulisatin (SI) on the intestinal transfer of glucose and tyrosine.* In ordinates, the µmol of glucose or tyrosine which enter (influx) or leave (efflux) the serosal compartment are indicated. Vertical bars show the standard error of the mean.

permanent inhibitory effect of oxyphenisatin observed *in vivo* after the withdrawal of oxyphenisatin and repeated washings of the intestine (8). It is plausible that this compound binds to the tissue, as occurs with phenolphthalein (1), thus changing the structural conformation of surface membrane proteins and, therefore, interfering with the transport mechanisms of the cell. Moreover, oxyphenisatin may interfere with cellular energy sources since this compound diminishes the oxygen consumption of the intestinal tissue (4).

When a high concentration of oxyphenisatin was placed only in the serosal, glucose transport was inhibited as the S/M ratio was reduced from 2.41 (controls) to 0.96 (10^{-3} M oxyphenisatin). However lower oxyphenisatin concentrations (10^{-5} and 10^{-7} M) showed no effect. These results, together with those obtained from preincubation experiments, suggest that the inhibitory activity probably takes place at the brush border of epithelial cells. This may explain the finding that only a high concentration of oxyphenisatin in the serosal is able to inhibit the sugar absorption as the compound needs to diffuse to the mucosal compartment through the muscular layers.

Present data support previous results *in vivo* (8) concerning the relation between the inhibitory effect and the presence of two free phenolic groups as well as the lack of inhibition elicited by the corresponding sulphuric ester derivates.

On the other hand the sensibility found *in vitro* is much higher than *in vivo* since *in vitro* 10^{-3} M oxyphenisatin exerts 100 % inhibition on glucose absorption while *in vivo* the same concentration results in only a 50 % inhibition.

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Resumen

Se ha estudiado el efecto que la oxifenisatina y otros tres derivados isatínicos ejercen sobre la absorción de glucosa y tirosina en el intestino delgado de rata, *in vitro*. Los dos derivados con grupos fenólicos libres inhiben marcadamente el transporte activo de ambos sustratos mientras que los que tienen grupos fenólicos esterificados con sulfato no ejercen ningún

efecto. La preincubación durante 1 min con oxifenisatina 10^{-3} M es suficiente para inhibir completamente la absorción de glucosa.

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